УДК 547

A. T. Lebedev,
T. Yu. Samguina

Organic Chemistry Department,
M. V. Lomonosov Moscow State University,
119991, Russia, Moscow

DE NOVO SEQUENCING OF AMPHIBIAN PEPTIDES AS POTENTIAL PHARMACEUTICALS OF FUTURE GENERATIONS

Keywords: modern mass spectrometry, complex biopolymers, taxonomy and biodiversity studies.

Modern mass spectrometry is the most powerful, sensitive, and informative tool for the identification and quantification of chemical compounds. It handles anything beginning from c of chemical elements and finishing with the most complex biopolymers. Nowadays mass spectrometry has become a key-method of bio-medical studies including proteomics.

Modern studies in the field of the diseases of the XXI century make the investigators look at the living representatives of flora and fauna in order to understand their mechanisms of immune defense and protection from the deteriorating environment and pathogenic micro organisms. Amphibians as one of the leaders of immune resistance live on the earth for hundreds millions of years. Their dorsal glands produce a cocktail of biologically active compounds, mainly peptides, which may successfully fight micro organisms and even predators. Skin secretion of amphibians contains wide spectrum antibiotic and neuro peptides, critical for the immune response and active at the levels of 10^{-9} M. They can also show antifungal and antiviral activities, stimulate insulin synthesis, inhibit no synthesis, and be analgesics. The mechanism of action of antibiotic peptides is completely different in comparison to that of the existing pharmaceuticals: amphipatic α-helix destroys phospholipid bilayer, leading to the lysis of the pathogenic cells. Since this mechanism prevents development of the pathogens resistance, antimicrobial peptides are very perspective pharmaceuticals of future generations.

Skin secretions of various frog species were obtained by mild electric stimulation. Their LC-ESI-MS/MS analysis was carried out with Thermo ICR and Orbitrap mass spectrometers (Thermo Scientific). CID, ECD, HCD, and ETD were applied in MS^2 and MS^3 modes to achieve the targeted sequence coverage. To sequence

SS-containing peptides crude secretions were preliminary reduced (DTT+ iodoacetamide) or oxidized with performic acid.

The developed *de novo* sequencing algorithm involves the analysis of three versions of original samples of the frogs' skin secretion: intact, carboxamidomethylated and oxidized ones. The combined analysis allows achieving complete sequence coverage of all frog peptides including long (up to 50 aa) ones. It resolves the problems of S-S bonds, cyclization of short peptides, the presence of isobaric (e.g. lysine/glutamine) amino acid residues in the sequence. An efficient approach of easy and reliable differentiation between isomeric Leu/Ile involves production and isolation of primary $z^{\cdot}$ ions, followed by radical site initiation of their fragmentation with formation of *w*-ions, characteristic of the isomeric amino acid residues. The resulting spectra are very selective with targeted *w* ions usually being the most abundant. Extracted ion plotting often applied in environmental tasks demonstrated its efficiency to detect all peptides related to a certain family. More than 200 new natural peptides were sequenced in terms of the present study. Their biological activity against microorganisms was studied. Thus activity of brevinin 1Tb measured with PMEU Spectrion® (Portable Microbe Enrichment Unit) technology appeared to be in the nanomole range, i.e. that of the modern antibiotics.

Peptidome representation with 2D-maps based on the simple mass spectrometry parameters shows itself as a very convenient method to distinguish frogs of closely related species, and making mass spectrometry a powerful tool for taxonomy studies. Moreover, the applicability of the proposed approach to differentiate the frogs of the same species but different populations was successfully demonstrated. It involves changes in the sequences of similar peptides due to diversity of natural habitat. The animals face different microbes and synthesize the most efficient peptides to fight them. Therefore, interspecies and intraspecies biomarkers revealed by mass spectrometry may be very helpful for future taxonomy and biodiversity studies.